DRAFT

United States
Department of
Agriculture

Forest Service

Forest Pest
Management

Davis, CA

STUDY PLAN

OFF-SITE MOVEMENT OF Bacillus thuringiensis SPRAY APPLIED IN COMPLEX FORESTED TERRAIN - 1992 PHASE

Healthy Forests
Make A World
Of Difference

FEBRUARY 1992

Pesticides used improperly can be injurious to human beings, animals, and plants. Follow the directions and heed all precautions on labels. Store pesticides in original containers under lock and key—out of the reach of children and animals—and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides where there is danger of drift when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment, if specified on the label.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U.S Environmental Protection Agency, consult your local forest pathologist, county agriculture agent, or State extension specialist to be sure the intended use is still registered.

STUDY PLAN
FEBRUARY 10, 1992

Off-site Movement of _Bacillus_ _thuringiensis_ Spray Applied in Complex Forested Terrain-1992 Phase

Cooperators:

USDA Forest Service
  Intermountain Region - FPM
  Washington Office - FPM
Utah Department of Agriculture
U.S. Army Dugway Proving Ground
National Weather Service

Prepared by:

John W. Barry
USDA Forest Service
Forest Pest Management
2121C, Second Street
Davis, CA 95616
(916) 758-4600

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PREFACE

This study plan covers field procedures to be followed in conduct of an off-site spray movement study during May-June 1992. The study will be conducted in Parleys Canyon and Lambs Canyon, Salt Lake County, near the Mountain Dell Golf Course in conjunction with the 1992 Utah gypsy moth eradication project. A biological pesticide *Bacillus thuringiensis* will be applied by helicopter. As of this writing no decision has been made on brand of *B.t.* to be used. The study is in follow-up to recommendations from Program WIND, (a U.S. Department of Agriculture-Forest Service (USDA-FS) and U.S. Army cooperative meteorological and computer model study); to recommendations from the USDA-FS national steering committees to further evaluate and technology transfer of computer models that predict the movement and deposition of sprays released from aircraft; and to the need for data on the environmental fate of Bt in the environment. The off-site movement study is a continuation of a 1991 study with similar objectives. Scientists from the USDA-FS and U.S. Army, in cooperation with Utah State Department of Agriculture; and National Weather Service; will participate in one or more aspects of this study. The aerially Bt applied spray, to be sampled during this study, will be sprayed by helicopter under operational conditions of the eradication project. Included in this project will be a study to investigate Bt background levels in the 1991-1992 Parleys Canyon treatment area. No special spray treatment will be applied and no tracers will be added to the spray tank mix for the benefit of this study. This study plan may be modified as needed and as agreed to by the cooperators. Results will be published in the open literature.

INTRODUCTION

Off-target movement of pesticides from forest spray operations has been a concern since aircraft were first used to spray trees (Neillie and Houser 1922). The concern primarily centers on potential environmental impact of pesticides on non-target species. Biological pesticides, such as Bacillus thuringiensis (Bt), are not exempt from this concern. Assessing potential environment impact of pesticides first requires quantitative data on the amount of pesticide that moves and deposits off the target site, followed by conducting environmental impact evaluations. Off-target movement also represents an inefficient use and economic waste of pesticides. For these reasons data are also needed to quantitate off-target movement that may lead to improving efficiency and efficacy of aerial spray operations. Predictions of the Forest Service Cramer-Barry-Grim (FSCBG) aerial spray model (Bjorklund et al. 1989), a computer model that predicts travel and deposition of aerial sprays, has been compared favorable to several sets of observed field data and reported by GCA Corporation (1971); Boyle et al. (1975); Dumbauld et al. (1976); Dumbauld et al. (1977); Rafferty et al. (1987), Rafferty et al. (1988), Rafferty et al. (1989), Teske et al., (1991), and Barry et al., (1992).

Sampling off-target drift of pesticides in forests and over complex topography presents technical challenges. Researchers have had relatively few opportunities to obtain such data in forested, complex terrain and few references are available in the literature. In situations where researchers have tried, results have been somewhat disappointing due to a variety of reasons including type of samplers and tracers used, sample contamination, and inadequate weather monitoring. Spray drift resulting from treatment of coniferous seed orchards has been reported by Barry et al. (1983); however the reported tests were conducted in relatively flat terrain. Rafferty et al. (1988) also reported deposition drift downwind to 2500 meters.

A similar study was conducted in the immediate vicinity of the proposed study site in 1991 (Barry et al. 1992). This is a continuation of the 1991 Study.

OBJECTIVE

The objective is subdivided into three tasks as listed below:

Task 1 - to quantitate off-site movement of Bt as measured by aerosol, impaction, and deposition samplers.

Task 2 - to compare FSCBG model predictions of air concentration (dosage and total dose) and deposition to observed data obtained from field samplers.

Task 3 - to investigate naturally occurring levels of Bt in the operational and study areas and to monitor levels resulting from 1991 and 1992.

SCOPE

The off-site movement study and treatment site is located in Salt Lake County, Utah, R2E, T1S, Sections 2,3,4,9,10, 11, 12, and 13. These sections, composed of public and private lands, are located in Parleys Canyon and Lambs Canyon, along Interstate 80. The terrain is mountainous and at lower elevations, and western and southern exposed slopes are partially covered at the lower elevations with Gambel oak. The site is ideally suited for this study due to topography, channeling of drainage winds, and physical access.

The treatment site, designated as the Miller Creek Spray Block (SL-1), consists of 8,846 acres (Figure 1). Parleys and Lambs Canyons are included in SL-1. It will be treated by a helicopter (Hughes 500D and/or Bell 206B-111) applying Bt in May-June 1992 to eradicate gypsy moth, a defoliator of oak and other deciduous trees. After the first spray is applied, treatment will be repeated two additional times at five day intervals thus providing an opportunity for three replicated trials. Off-site movement studies will be conducted only in the vicinity of Block SL-1. Spray moving down slope of the treatment area will be sampled by a variety of samplers positioned downwind to approximately 3 miles. Surface weather will be monitored by three weather stations and upper air by accoustical sounder. Several organizations will cooperate in this study. A dry run will be conducted on or about May 15, 1992 to brief field crews, to practice field procedures, and to coordinate timing.

METHODS-AEROSOL AND DEPOSIT SAMPLING

Application

The SL-1 block of acres will be treated operationally with Bt pesticide undiluted applied by helicopter at 0.5 gallons per acre. A total of 4,423 gallons will be applied; however not all material will be applied in the canyons that drain the airshed to be studied. Success of the study is dependent upon an organized drainage wind that results from nighttime cooling of slopes. To increase potential for a successful study the Treatment Supervisor's will be requested to:

1. Begin each of the 3 applications of SL-1 at first light when the pilots believe it is safe to fly and complete spraying before upslope winds begin.

2. Avoid spraying SL-1 if cloud cover precludes surface cooling. Drainage wind depends upon surface cooling that might not occur if clouds hold warm air near the surface.

3. Complete spraying in SL-1 before up-canyon winds develop. It would be ideal if Lambs Canyon and the area near Parleys Canyon were treated at first light.

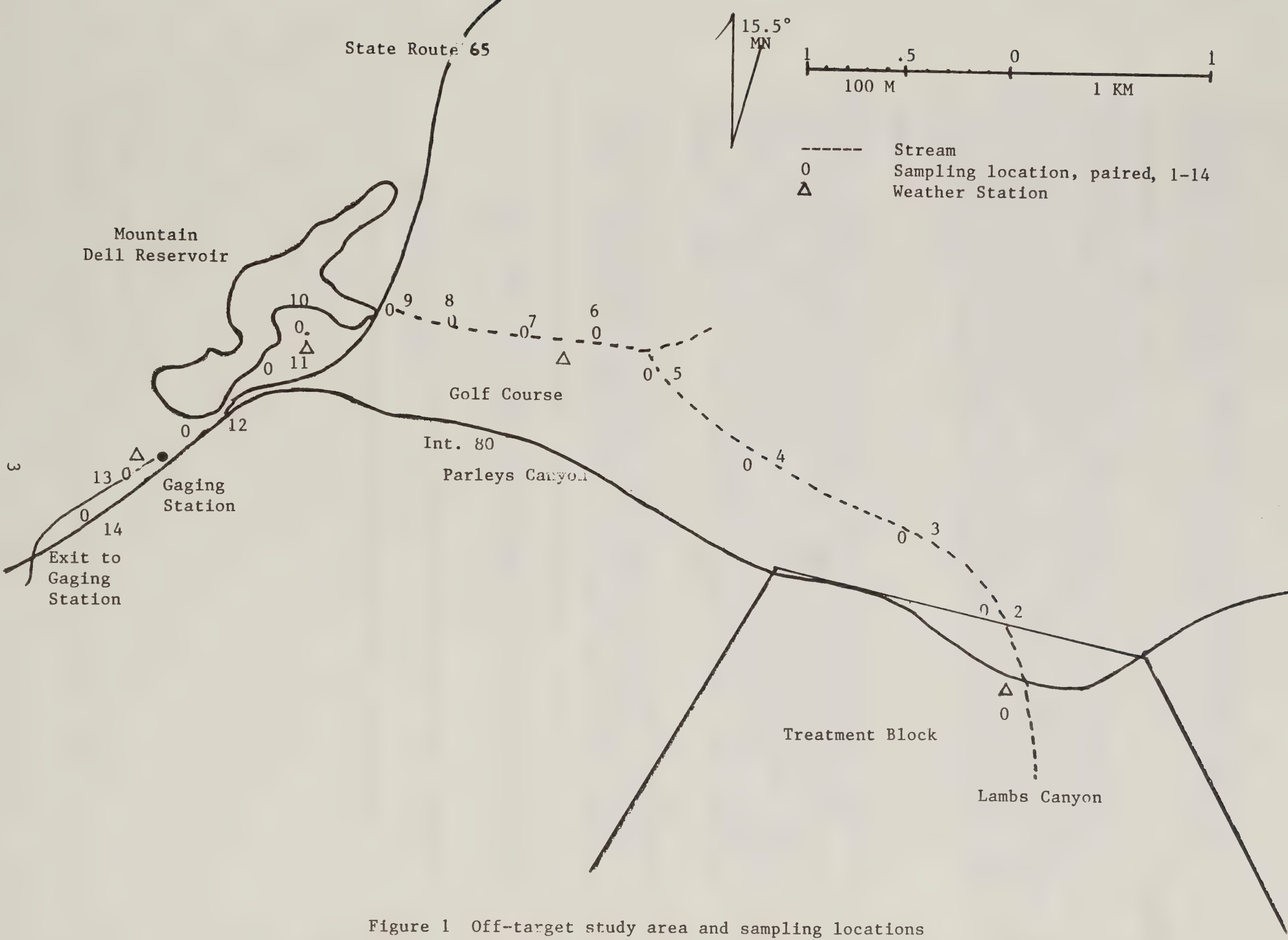

Figure 1 Off-target study area and sampling locations

Field data collection requirements are listed in the paragraph Field Data Requirements. It is requested that the information listed be provided each day that Block SL-1 is treated.

Spray Material (Tank Mix)

The material to be applied (see attached pesticide label in Appendix) is a commercial formulation of *Bacillus thuringiensis*. Berliner var. Kurstaki, with a potency of 12,600 infectious units per milligram equivalent to 48 billion international units (BIU) per gallon. It will be applied undiluted at the rate of 0.5 gallons (24 BIU) per acre. No tracers or other additives will be added to the tank mix. The material has a relatively low rate of volatility. Wind tunnel testing of the atomization under conditions (atomizer, air speed, and application rate) approximating the anticipated operational conditions, is provided in Table 1.

Sampling

Off-site movement of Bt will be sampled downwind to approximately 5 kilometers for air concentration with the Wagner sampler (Figure 2) aspirating and "U"-shaped brass Rotorod samplers (Figures 3-4-5), rotating; for impaction with the "U"-shaped brass Rotorod[1] static (not rotating); and for deposition with white Kromekote cards and Mylar sheets. Other types of samples may be used if necessary to meet study objective. Twenty-eight sampling stations (14 pairs) will be positioned at 14 locations along a line that follows the drainage down-canyon from Lambs Canyon. Approximate location of the sampling stations is shown in Figure 1.

Samplers will be positioned and set-out the morning of treatment, and picked-up after the spray operations and spray cloud passage is complete. The Test Officer in consultation with the Project Meteorologist will decide when to activate and deactivate the samplers to insure sampling the entire spray cloud.

Each sampling station (Figure 6) will consist of the following:

1. 4 each Wagner samplers, operated sequentially (one at a time) at 12.5 liters of air per minute (lpm) with a 12.5 critical orifice installed in the vacuum lines.
2. 2 each "U"-shaped Rotorods rotated clockwise at 2400 rpm, potentially sampling 120 (lpm).
3. 2 each "U"-shaped Rotorods positioned statically (not rotating).
4. 2 each Mylar sheets.

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[1] *Rotorod^R is a registered trademark of Metronics Associates, Inc.

The Wagners and Rotorods will be elevated at 1.5 meters above the ground, and the Kromekote cards and Mylar sheets will be placed in plastic holders and in-turn these will be placed on boards at ground level. The board will insulate the samplers from moisture and help to reduce shielding from plants. The samplers will be activated prior to anticipated spray cloud arrival. Further details will be coordinated with the U.S. Army Dugway Proving Ground (DPG) designated Test Officer.

MEMBRANE FILTER HOLDERS

Purpose: To hold filter media.

General Description: Several holders have been designed to support membrane filters and other sheet fiber materials for sampling airborne particles. Most holders consist of a cylindrical body, one end of which is adapted for connection to a vacuum source. The other end is fitted with a circular supporting screen or carbon pad. A clamping device seals the filter between the supporting screen and an inlet cup to prevent bypassing of the sampled aerosol. When the inlet cup is fitted with an adapter, samples may be taken in a closed system or from an air duct. One type of holder (fig. 45) is designed to accommodate both the unringed and the ringed membrane filter (filter mounted on a plastic ring). The plastic ring holds the filter against the supporting screen and eliminates the need for an inlet cup. The materials of construction and sealing devices vary with designs.

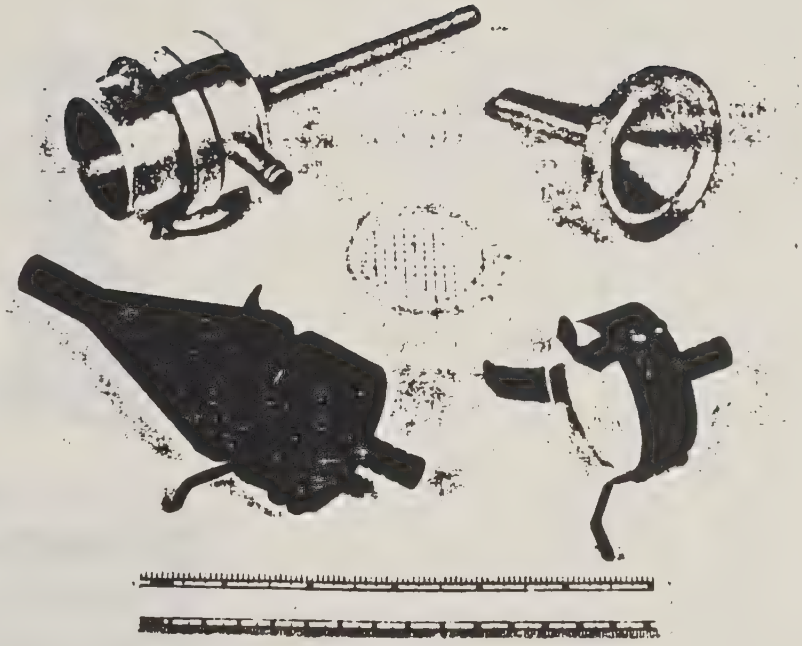


Figure 45.

Sources: Millipore Filter Corp., 36 Pleasant Street, Watertown 72, Mass. Gelman Instrument Co., Chelsea, Mich. Drawings of the membrane filter holder (fig. 45) available from Technical Development Laboratories, Communicable Disease Center, U.S. Public Health Service, P.O. Box 769, Savannah, Ga.

Reference: 76.

From: Wolf, H.W., P. Skaliy, L.B. Hall, M.M. Harris, H.M. Decker, L.M. Buchanan, and C.M. Dahlgren. 1959. Sampling Microbiological Aerosols. Washington: Public Health Service.

Figure 2. Membrane filter holders similar to the Wagner sampler.

Figure 3. Basic Rotorod sampler motor.

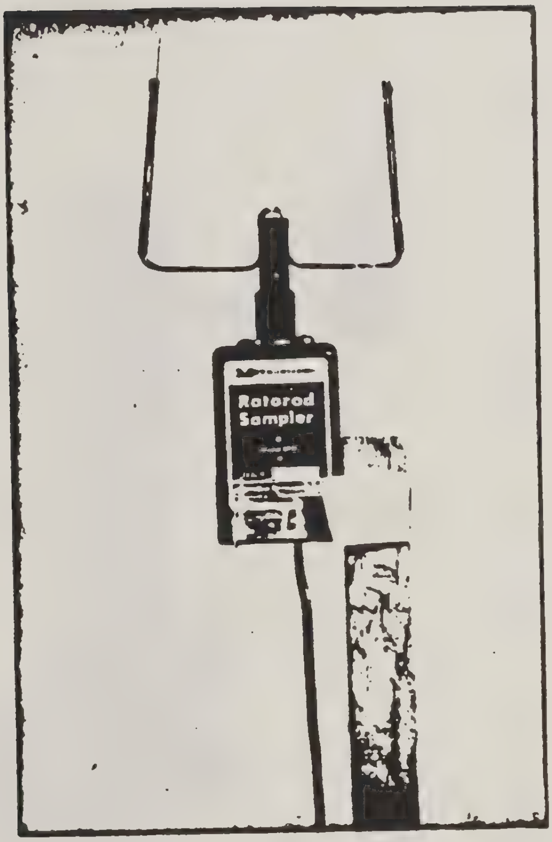


Figure 4. Field mounted Rotorod sampler - taped. The Rotorod sampler can be temporarily fastened with glass filament tape to any convenient stake or post.

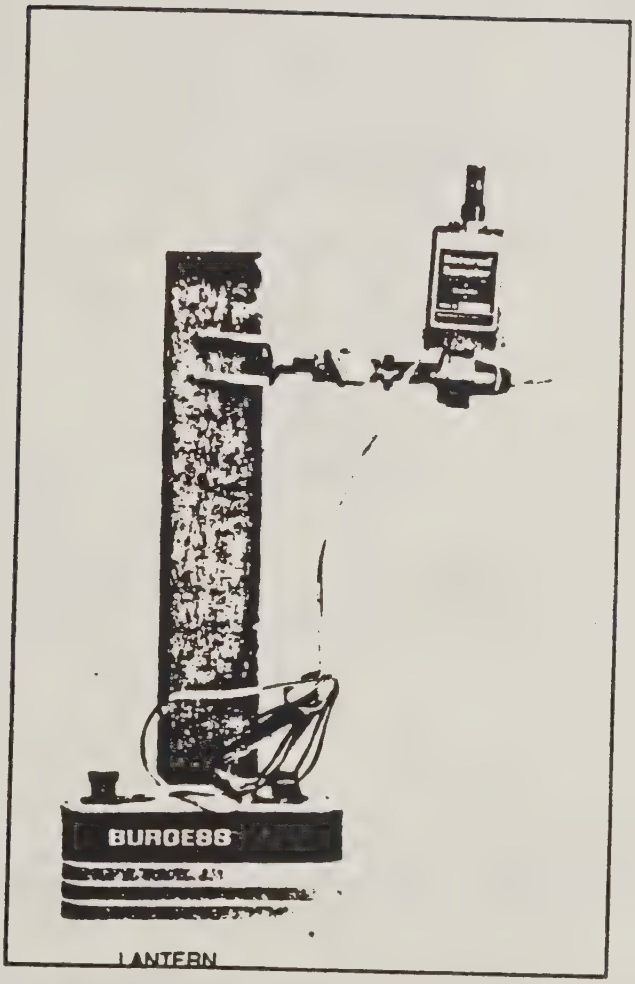
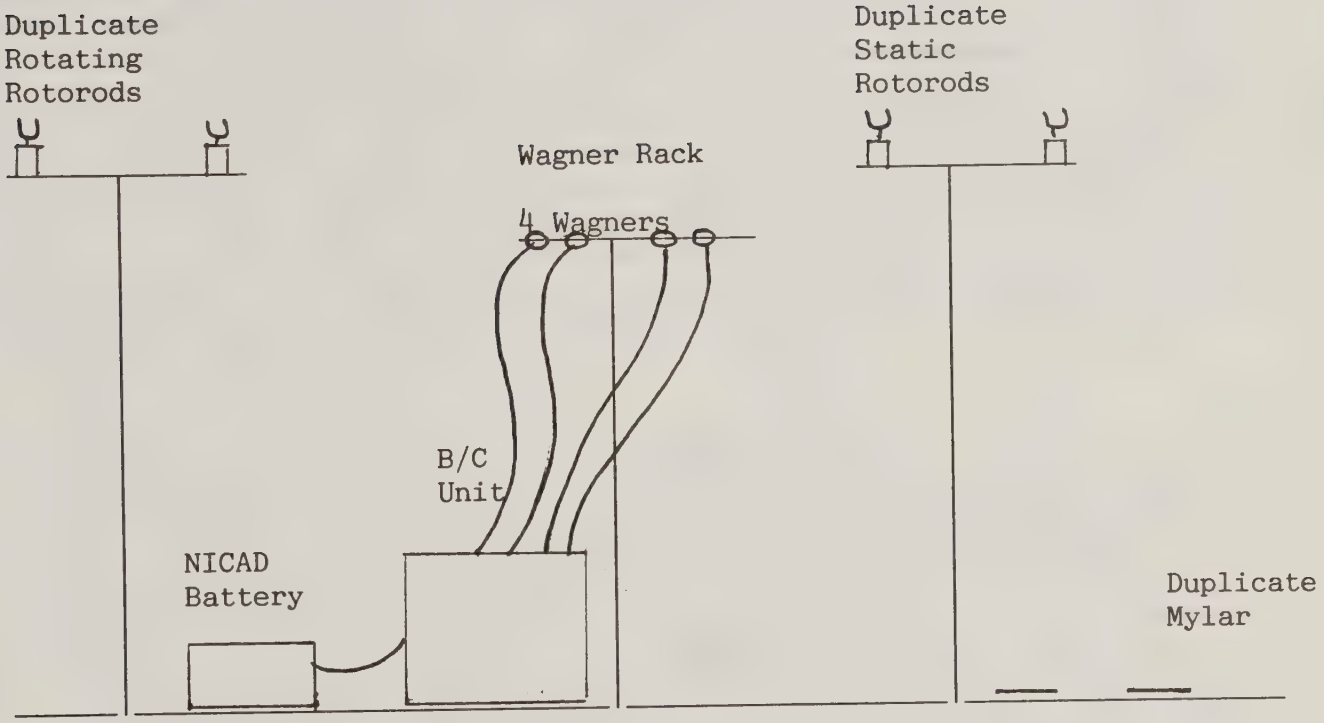


Figure 5. Rotorod sampler powered by 12-volt battery.



Notes: Samplers (Wagner, Rotorods, and Mylar) at each station should be positioned perpendicular to axis of the spray cloud to avoid one sampler apparatus shielding the next.

Static Rotorods arms will be oriented perpendicular to drainage wind

Wagners opening will point to ground. First Wagner will be a control and last Wagner turned-on after cloud passage.

B/C=Biological/Chemical sampling system that contains vacuum pump, power unit, radio receiver, sequencers, and control panel.

Figure 6. Diagram of a sampling station.

The Test Officer log and report will record when each sampler is set out, picked up, activated, and deactivated. It also will record all malfunctions and missing samplers.

Wagner Sampler

The Wagner sampler is similar to the holders shown in Figure 2. The Wagner supports a synthetic membrane filter, backed by a wire screen on the down flow side. Specific type of filter will be determined by the Laboratory Officer. Four Wagners will be used at each sampling station, the first and last Wagner should have negative counts indicating that the entire spray cloud passage was sampled. Combined the counts will provide a total dose. The Wagner will sample **12.5 liters of air per minute**, supported by the DPG B/C sampling unit that contains vacuum pumps, and control switches (Figure 7). The vacuum lines will have a 12.5 critical orifice in line. The Wagner opening will be pointed toward the ground. The unit will use 2 each 12 volt NICAD batteries. The B/C units will be operated manually or remotely by wire at the direction of the Test Officer.

Rotorod^{R} Sampler

The "U"-shaped brass Rotorod sampler, developed by Metronics and currently produced by Ted Brown Associates, is a rotating arm impaction device capable of obtaining quantitative data of airborne particulates in the size range $\geq$ 10 microns. At a nominal 2400 rpm which moves the collecting surfaces through the air and thus causes particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. It samples 120 liters per minute when rotating at 2400 rpm with a 100% collection efficiency according to Edmonds (1972). The collecting surface of the "U"-shaped rod is 0.159 cm. Its basic components are a constant speed motor and aerodynamically designed collector rods which are rotated by a 12-volt motor (Figure 3). The reference (Ted Brown Associates, 1976 and Edmonds, 1972, and Flottum, 1984) describe the sampler and provide instructions for its installation, operation and evaluation.

The "U"-shaped Rotorod samplers will be used in pairs at each sampling station. The Rotorod will be rotated to sample airborne Bt particles through impaction. Rotorods will also be used statically to collect particles by impaction caused by moving air. All Rotorods will be elevated 1.5 meters above ground.

The Rotorods will be used in duplicate at each station, 2 rotating and 2 static. The rotating Rotorods will be connected to a 12-volt motor and powered by a 12-volt Burgess or comparable battery. The Rotorods will be operated during the same time as the Wagner samplers.

[illegible] similar [illegible] shown [illegible]
[illegible] filter, [illegible]
[illegible] type of filter will be [illegible]
[illegible] at each sampling [illegible]
[illegible]
[illegible] the [illegible] will [illegible]
12.5 [illegible] air [illegible] minute [illegible]
[illegible] vacuum gauge, and control [illegible]
[illegible] 12.5 [illegible]
[illegible] The unit will use [illegible] battery
[illegible] rated manually [illegible]

shaped brass Rotorod sampler, developed by [illegible]
Ted Brown Associates [illegible]
[illegible] data [illegible] particles
[illegible] 2400 rpm [illegible]
[illegible] particles within the [illegible]
[illegible] to become impacted [illegible]
[illegible] liters per [illegible]
efficiency according to [illegible]
[illegible] 154 cm [illegible]
[illegible] collection [illegible]
[illegible] Figure 5 [illegible]
[illegible] 1964) describe the [illegible] and provide [illegible]
[illegible] and evaluation.

[illegible] Rotorod samplers will be used in pairs at each [illegible]
[illegible] Rotorod will be rotated to sample [illegible] particles through
[illegible] also be used [illegible]
[illegible] moving air. All Rotorods will be [illegible] 1.5 [illegible]

[illegible] at each station. [illegible]
[illegible] will be connected to a [illegible]
[illegible] Rotorods will be [illegible]

Figure 7. B/C sampling unit with NICAD battery, vacuum tubing, Rotorod, and Wagner sampler.

Mylar Sheet

Mylar sheet, measuring 4 5/16 x 6 9/16 inches also will be positioned at ground level in duplicate near the Kromekote cards. The Mylar will also collect deposition resulting from gravitational settling. Mylar will be provided by the USDA Forest Service.

Control Samples

Field control samples will be used in addition to laboratory controls specified in laboratory standard operating procedures. Controls will be set up and operated (Wagners aspirated and Rotorods rotated) for 10 minutes at every numbered sampling station. Wagner samplers are sterilized in the laboratory and the ends plugged with cotton. The cotton must be removed before aspirating samplers. This will be done when the sampling station is set-up and before spray release begins. Controls will be packaged and removed from the sampling area prior to spraying. A Mylar sheet control sampler also will be placed at every even numbered sampling station and picked up prior to commencement of spraying.

Quality Control

Prior to the study the DPG Laboratory Officer will evaluate a fresh sample of the Bt to be sprayed to become familiar with its physical and biological properties to the extent these factors might influence laboratory analyses. The Laboratory Officer will also evaluate suitability of two growth media recipes provided in the Appendix. Applicable DPG standard operating procedures will be followed including quality control and use of control samples. Data report should include results of laboratory control samples. Quality control includes both handling and exposure of control samplers and samples to detect Bt background, natural and accidental contamination of samples by Bt.

Review of the 1991 laboratory assay data suggest need for improved quality control in the field. Field personnel should take special care to:

1. **Need** to pick-up all equipment needed for the trial;
2. **Need** to remove cotton plugs from intake of Wagner sampler;
3. **Need** to locate samplers to avoid golf course sprinkling;
4. **Need** to report all discrepancies e.g. plugged samplers; and
5. **Need** to follow suggested sampler marking system per Study Plan.

A liter of tank mix that represents the batch of Bt applied to SL-1 will be collected from the spray aircraft by the Air Operational Officer and sent to the DPG laboratory for analyses. The sample should be placed in a plastic bottle and double packaged in plastic bags. The sample should be dated and Bt lot number placed on label.

Avoiding Contamination

The most serious threat to the integrity of the sampling is contamination of samples with Bt. Bt is a spore former that occurs naturally in the soil. Being a spore former it is persistent as opposed to vegetative cells that are susceptible to UV radiation and other degrading factors. After treatment the study area including foliage , soil, and other surfaces will be contaminated. Potential sources of sampler contamination include contaminated equipment, (sampling equipment, vehicles, containers) non-sterile samples, secondary aerosols (natural and man made), improper handling, packing, transportation of samplers, and contaminated crews (skin and clothing). Potential of contamination beginning of first trial contamination of equipment from DPG is minimal as Bt has not been used on the DPG ranges nor in their laboratories. But once Bt is released contamination is a serious threat. This applies to sampler pick up after the first trial. Suggested procedures to reduce potential for contamination include:

1. Sterilize samplers - Wagners and Rotorods.
2. Label samples in accordance with plan.
3. Wear clean clothing daily.
4. Avoid creating dust and secondary aerosols near samplers.
5. Approach samplers on downwind side.
6. Scrub Mylar card holders to remove any Bt contamination.
7. Handle Mylar with sterile instruments (e.g. forceps) or clean gloves remembering once the exposed Mylar is touched the instrument or glove is contaminated.
8. Follow laboratory officers instructions on handling and transporting samplers.
9. Keep vehicles and personnel upwind during trials - avoid the spray cloud.
10. Wash-down vehicles, B/C units, batteries, racks, and tote boxes. This will help to reduce potential of contamination from secondary aerosols and cross contamination.
11. If Rotorods are accidentally contaminated by dropping on ground, touching with contaminated hands or gloves, etc. this should be reported to the Test Officer.

Sampler Marking

Sampler marking codes will be used throughout the study - that is from the laboratory where the sampler is prepared through to the reporting of data.

a. **Wagner**

```
1   ----  1   ----  1   ----   W  ----
|         |         |          |
Trial     |         |          |
(1-3)     |         |          |
        Station     |          |
        (1-28)      |          |
                  Sequence     |
                    (1-4)      |
                             Wagner
                             Sampler
```

Wagners marking code will be placed on tape stuck to the Wagner.

b. **Rotorod (Spinning)**

```
1   ----  1   ----  RR  ----  A, B
|         |         |          |
Trial     |         |          |
(1-3)     |         |          |
        Station     |          |
        (1-28)      |          |
                  Rotorod      |
                  Rotating     |
                              Duplicate 1 use an A
                              Duplicate 2 use a B
```

Marking codes will be placed on outside of the Ziploc bag and not directly on the Rotorod.

c. **Rotorod (Static)**

```
 1   ----  1   ----  RS  ----  A, B
 |         |         |          |
Trial      |         |          |
(1-3)      |         |          |
          Station    |          |
          (1-28)     |          |
                    Rotorod     |
                     Static     |
                               Duplicate 1 use an A
                               Duplicate 2 use a B
```

d. **Mylar Sheet**

```
 1   ----  1   ----  M   ----  A, B
 |         |         |          |
Trial      |         |          |
(1-3)      |         |          |
          Station    |          |
          (1-28)     |          |
                    Mylar       |
                                |
                               Duplicate 1 use an A
                               Duplicate 2 use a B
```

Marking codes will be placed on the outside of the container used to collect the Mylar and not directly on the Mylar.

Sampling Equipment Requirements

There will be 14 duplicate sampling stations numbered 1-28 (Figure 6). The duplicates will be approximately at the same distance downwind but should be separated from 20 to 50 meters from each other. Samplers and samples from duplicate stations will be considered duplicates as paired duplicates in the analyses. DPG will provide all equipment and samplers except the Forest Service will provide:

Equipment (Per Trial)

	Required	Controls	Spare
12.5 lpm Critical in-line orifice	112	--	3
B/C Sampling Units w/Wagner rack	28	--	3
NICAD battery	56	--	5
Rotorod motors w/bracket	56	--	10
Stands for Wagners and Rotorods	28	--	2
Mylar card holders	56	--	10
Burgess 12-V Battery (unless powered by B/C)	56	--	20

Samplers (Per Trial)

	Required	Controls	Spare
Wagners	112	20	12
Rotorods spinning	56	20	12
Rotorods static	56	20	12
Mylar sheet	40	10	12

Laboratory Assay

Wagners. Membrane filter will be dissolved in an appropriate fluid, and diluted and plated out on an appropriate growth media to be determined by the DPG Laboratory Officer. Data will be reported by colonies per sample. Excess collecting fluid will be retained for additional assay as required and possibly for assay at another laboratory.

Rotorods. Rotorods will be retrieved from the motor by covering the Rotorod with a Ziploc bag and sealing the zip without directly touching the rod. Labels will be placed on the outside of the Ziploc bag and not on the Rotorod. In the DPG laboratory the Bt will be extracted from the Rotorod and the Ziploc bag, and the diluent will be diluted and plated. Excess collecting fluid will be retained for additional assay as required.

Mylar Sheets. Mylar sheets will be retrieved in the field by sterile forceps and placed in sterile glass bottles or Ziploc bags. Bt will be extracted and plated-out in the same manner as the Rotorods at the DPG laboratory. Bt deposition could be high at those stations closest to the treatment block.

Laboratory data should be provided to Study Director by July 15, 1992.

Weather Instrumentation and Measurements

Four solar powered weather EMCOT stations or comparable (Ekblad et al., 1990) will be used to collect wind speed, wind direction, temperature, and relative humidity. Sensors will be positioned at 4 feet and 20 feet levels. Specifications of EMCOT include:

- Capable of being erected by one person
- Transportable in a sedan or pickup
- Real time screen display
- High frequency data, greater than 2/second
- Store 8 hours of data
- Display events graphically on the computer screen or printer
- Two temperature sensors
- Relative humidity sensor
- Fast response vertical wind speed
- Horizontal wind speed and direction

The weather stations will be deployed at strategic locations within the study area. Wind data will be collected at 2-second intervals to provide turbulence data by the FSCBG model. Data collection will begin 15 minutes prior to beginning of spraying and continue for two hours after spraying.

Specific location of the three stations will be decided upon consultations with project meteorologist.

The stations will be installed, operated, and maintained by an engineering technician from the USDA-FS Missoula Technology Development Center (MTDC).

MTDC will provide computation of all weather data in a data report by July 15, 1991.

Field Data Requirements

Data requirements and person responsible for providing the data are listed below. These data will be needed for each of the three treatments of Block SL-3.

1. Operational Treatment Data R-4

    Date
    Aircraft
    Pilot(s)
    Time block treatment began
    Time block treatment ended
    Total gallons applied
    Total acres treated
    Description of block treatment - specifically where the spray swaths were applied and when.
    Other remarks from pilot

2. Map of sampling stations and EMCOT weather stations showing measured location (Test Officer)

3. Weather data from 4 stations collected at 2 heights (2 meters 6.5 meters) DPG and NWS

    Wind speed
    Wind direction
    Temperature
    Relative humidity
    Cloud cover
    Barometric pressure (from National Weather Service, Salt Lake City airport)

4. Sampling (DPG Test Officer)

    Time samplers set out
    Time samplers picked-up
    Time samplers were activated
    Time samplers were deactivated
    Time control samplers were activated, and deactivated, set out, and picked up
    Sampler malfunctions

DATA ANALYSES

Data analyses, initially, will approach each task separately, and as appropriate integrate results and analyses. Data from the laboratory and field controls will be analyzed and considered in the analyses and discussed in the report. Duplicate samplers have been included in the design to increase confidence as field sampling is recognized as being inherently highly variable. A statistician will be contracted to analyze these data and to assist in preparing the data analyses section of the report and manuscript.

Task 1. Laboratory results will be compared and analyzed to determine a level of confidence for the types of samplers used and the resulting samples. Analyses will address questions to include: are recoveries within expectations and model predictions for given observed weather conditions and downwind distances; are recoveries relatively consistent; are the Wagner recoveries more or less consistent than the Rotorod recoveries; and what are the differences (significant) among duplicates at same station and same downwind distances for the Wagner and Rotorods? The Kromekote card will be evaluated qualitatively only, as recoveries are dependent upon observing stains left by depositing spray drops. Positive cards are not expected at distances greater than 0.5 miles from the downwind edge of the spray block. Deposition on the Mylar sheets will be evaluated statistically by comparing duplicate recoveries as a function of downwind distance, and to FSCBG model predictions. The Mylar assay is more sensitive thus positive recoveries are expected at most stations.

Task 2. Analyses under Task 2 will overlap that of Task 1 but focus on quantitative data and provide statements of statistical confidence in the quantitative recoveries.

Task 3. FSCBG model runs will be made after the field studies are completed. Input to the model will be the conditions existing during spraying supplemented by estimates of non-measured conditions e.g. height of the mixing layer and winds at spray release height. A statistical analyses of predictions among trials will be made along with significant differences that might be noted when input parameters are changed or modified during the FSCBG sensitivity analyses.

COORDINATION

Treatment Supervisor - John Anhold (801) 625-5292, FTS 586-5292
Responsible for overall conduct of the eradication program and providing manpower as requested.

Public Affairs Officer (PAO) - L.J. Western (801) 524-6207, FTS 588-6207
Responsible for all public affairs activities to include press releases, media contacts, public inquiries related to the program and studies, and coordination with Dick Whitaker, DPG PAO.

Study Director - Jack Barry (916) 758-4600, FTS 460-1715
Responsible for planning, coordination, documenting and reporting of the off-site spray movement study.

DPG Scientist - Bruce Grim (801) 831-3371
Responsible for all coordination administration and support with U.S. Army Dugway Proving Ground to include coordination between PAO and Dick Whitaker, Dugway public affairs officer and U.S. Army Aberdeen Proving Ground; and coordination with Dugway's Lockheed contract.

DPG Project Officer - Gary Sutton (801) 831-5638
Responsible for coordination with Lockheed contractor for Test Officer and field crews.

DPG Laboratory Officer - Lloyd Larsen (801) 831-5173
Responsible for preparation of samples, laboratory assay of samplers, quality control procedures, and reporting data.

Test Officer - Todd Warr (801) 831-5335
Responsible for set-up operation, and pick-up of sampling equipment, and quality control, and reporting on field operations.

Utah State Liaison Officer - Mark Quilter (801) 538-7190
Responsible for liaison, coordination between State and local jurisdictions, and study personnel.

Air Operations Officer - Andy Knapp (208) 364-4222
Responsible for providing data requested in paragraph 1 of Field Data Requirements.

SAFETY

Safety is everyone's responsibility both in practice and in reporting real and potential hazards. All personnel involved in this study will be familiar with and observe procedures outlined in the Operational Project Safety Plan. Supervisors are responsible to insure that personnel read the Safety Plan and all personnel are responsible for safe work practices. The primary safety hazard is driving on Interstate 80, particularly access and egress; travel on unimproved roads; operating vehicles and equipment during early morning conditions; and lifting of equipment. Weather stations will be located to avoid electrical wires and vehicle and foot traffic. Guy wires and stakes will be marked with fluorescent engineering tape. Weather stations may be fenced if safety and security is deemed to be a problem. The material safety data sheet and pesticide label for Bt are in the Appendix.

REPORTING AND TECHNOLOGY TRANSFER

Results of this test and the analyses will be reported in a joint USDA Forest Service/U.S. Army report. If the field and laboratory procedures are successful, a manuscript will be prepared and submitted to the American Society of Agricultural Engineering or the Journal of Applied Meteorology for publication. In addition, a paper will be offered for presentation to the summer or winter meeting of the American Society of Agricultural Engineers. Applicable results will be incorporated in FS training sessions directed at persons who develop environmental impact studies, and who plan and conduct aerial and ground spray operations.

BIBLIOGRAPHY

Barry, John W. 1991. Study plan off-site movement of *Bacillus thuringiensis* spray applied in complex forested terrain. USDA Forest Service, Forest Pest Management, Davis, CA.

Barry, J.W. and R.B. Ekblad. 1983. Forest Service spray drift modeling. ASAE Paper 83-1006, ASAE Summer meeting Bozeman, MT.

Barry, J.W., R.B. Ekblad, P.A. Kenney and L.A. Barber. 1983. Drift from aerial application to coniferous seed orchards. ASAE Paper AA-83-003, ASAE Joint Technical Session, Reno, NV.

Bjorklund, J.R., C.R. Bowman, and G.C. Dodd. 1989. User Manual - Forest Service aerial spray computer model FSCBG (PC) USDA Forest Service, Davis, CA.

Boyle, D.G., J.W. Barry, C.O. Eckard, W.T. Taylor, W.C. McIntyre, R.K. Dumbauld and H.E. Cramer. 1975. DC-7B aircraft spray system for large-area insect control, U.S. Army Dugway Proving Ground Report No. DPG-DR-C980A, Dugway, UT.

Ted Brown Associates. 1976. Operating Instructions for the Rotorod Sampler, Tech Manual No. 6. Ted Brown Associates, 26338 Esperanza Dr., Las Altos Hills, CA 94022.

Dumbauld, R.K., J.E. Rafferty and H.E. Cramer. 1976. Dispersion deposition from aerial spray releases. In: Proceedings of the *Third Symposium on Atmospheric Turbulence, Diffusion and Air Quality*, Raleigh, NC. (Reprint available from American Meteorological Society, Boston, MA).

Dumbauld, R.K. and J.E. Rafferty. 1979. Prediction of spray behavior above and within a forest canopy. In: Proceedings of the *Fourteenth Conference on Agriculture and Forest Meteorology*, April 2-6, 1979, Minneapolis, MN.

Dumbauld, R.K. and J.R. Bjorklund. 1977. Deposition profile calculations for the State of Maine 1977 spray program. H.E. Cramer Company, Inc. Technical Report TR-77-310-01, prepared for Litton Aero Products, Woodland Hills, California and the State of Maine Bureau of Forestry, Augusta, ME.

Edmonds, R.L. 1972. Collection efficiency of Rotorod samplers for sampling fungus spores in the atmosphere. Plant Disease Reporter, Vol. 56-8, pp 704-708.

Ekblad, R., Windall, K., and Thompson, B. 1990. EMCOT Weather Station. USDA Forest Service, Missoula Technology Development Center, Missoula, MT.

Flottum, P.K., D.C. Robacker, and E.H. Erickson, Jr. 1984. A quantitative sampling method for airborne sweet corn pollen under field conditions. Crop Science, Vol. 24., pp 375-377.

GCA Corporation. 1971. Model estimates of the deposition of aerial spray on a forest canopy, Technical Note. Contract No. DAA-D09-71-C-0003 with U.S. Army Dugway Proving Ground, Dugway, UT.

Geary, J.V., C.R. Bowman, J.E. Rafferty, and R.K. Dumbauld. 1987. Application of the FSCBG version 2 aerial spray model in forestry. In: Proceedings of Symposium on the Aerial Application in Forestry, October 20-22, 1987. Ottawa, Canada.

Neillie, C.R. and Houser, J.S. 1922. Fighting insects with airplanes. In: The National Geographic Magazine, XLI, 332-338.

Rafferty, J.E., John M. White, James F. Bowers, and John W. Barry. 1988. Comparison of program wind phase III deposition measurements with FSCBG 2 model predictions. DPG Document No. DPG/TA-89-007. U.S. Army Dugway Proving Ground, Dugway, UT.

Rafferty, J.E., J.M. White, J.F. Bowers and R.K. Dumbauld. 1989. Forest spray dispersion study: comparison of measured deposition with FSCBG2 model predictions. In: Proceedings of the American Meteorological Society Conference on Agricultural and Forest Meteorology, (pp 200-203). Charleston, SC.

Rafferty, J.E., R.K. Dumbauld, H.W. Flake, Jr., J.W. Barry and J. Wong. 1981. Comparison of modeled and measured deposition for the Withlacoochee spray trials. Report No. 81-3 under Contract 53-91S8-9-6260. USDA Forest Service, Forest Pest Management, Davis, CA.

Teske, M.E., K.P. Bentson, R.E. Sandquist, J.W. Barry and R.B. Ekblad. (In Press August 1991). Comparison of FSCBG model predictions with heather seed orchard deposition data. Journal of Applied Meteorology.

Wolf, H.W., P. Skaliy, L.B. Hall, M.M. Harris, H.M. Decker, L.M. Buchanan, and C.M. Dahlgrem. 1959. Sampling Microbiological Aerosols. Washington: U.S. Public Health Service.

ical Note. Contract No. LSA- [illegible]
Diego, Calif.

J. B. Heffernan and R.K. [illegible]
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F. Benneson, R.S. [illegible]
1971). [illegible]
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[illegible] M. Harris, [illegible]
Sampling No. 103 [illegible]

APPENDICES

1. Material Safety Data Sheet
2. Pesticide label
3. Recipe for media to culture Bt (From David Hobbs, NOVO)
4. Recipe for media to culture Bt (From Roy Beckwith)
5. Reprint - Collection Efficiency of Rotorod Samplers for Sampling Fungus Spores in the Atmosphere by Robert L. Edmonds
6. Reprint - A Quantitative Sampling Method for Airborne Sweet Corn Pollen Under Field Conditions by P.K. Flottum, et al.
7. Field Crew Check Sheet and Report
8. Assay Procedures for Biological Simulants/Sampler Preparation (MT-L389, 2nd Revision, 20 October 1987)

Novo Laboratories, Inc.
33 Turner Road
Danbury, CT 06810-5101

Emergency Phone Number: (203) 790-2600
Chemtrec Number: (800) 424-9300

MATERIAL SAFETY DATA SHEET

DATE: May 5, 1988

REVIEWED: 5/88

I. IDENTIFICATION

PRODUCT NAME: Foray 48B™

CHEMICAL NAME: *Bacillus thuringiensis Berliner* var. *kurstaki*

FORMULA: NA

CHEMICAL FAMILY: Biological insecticide

MOLECULAR WEIGHT: NA

SYNONYMS: NA

DEPARTMENT OF TRANSPORTATION:

HAZARD CLASSIFICATION: None

SHIPPING NAME: None

FREIGHT CLASSIFICATION: Insecticides, Agricultural, Liquid N.O.I.

CAS NUMBER: 68038-71-1

CAS NAME: *Bacillus thuringiensis Berliner* var. *kurstaki*

II. PHYSICAL DATA

BOILING POINT, 760 mm Hg: NA

SPECIFIC GRAVITY (H_2O = 1): 1.16

VAPOR DENSITY (AIR = 1): NA

PERCENT VOLATILES BY VOLUME: NA

APPEARANCE AND ODOR: Flowable liquid concentrate, characteristic fermentation aroma

POUR POINT: NA

pH: 4.0 - 4.5

VAPOR PRESSURE AT 20°C: NA

SOLUBILITY IN WATER, % BY WT.: Suspendable

EVAPORATION RATE (BUTYL ACETATE = 1): NA

III. HAZARDOUS COMPONENTS

MATERIAL	%	TLV (Units)	HAZARD
Bacillus thuringiensis Kurstaki	15	None	None known

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (TEST METHODS): NA

FLAMMABLE LIMITS IN AIR, % BY VOLUME: NA

EXTINGUISHING MEDIA: No special requirements

SPECIAL FIRE FIGHTING PROCEDURES: No special requirements

UNUSUAL FIRE AND EXPLOSION HAZARDS: None known to exist

V. HEALTH HAZARD DATA

TLV AND SOURCE: None established by ACGIH or OSHA

ACUTE EFFECTS OF OVEREXPOSURE

SWALLOWING: None known

SKIN ABSORPTION: Not known to occur

INHALATION: None known to exist

SKIN CONTACT: None known to exist

EYE CONTACT: None known to exist

CHRONIC EFFECTS OF OVEREXPOSURE

Repeated exposure via inhalation can result in sensitization and allergic response in hypersensitive individuals.

CARCINOGENICITY:
NTP? No
IARC Monographs? No
OSHA Regulated? No

EMERGENCY AND FIRST AID PROCEDURES

SWALLOWING: Rinse mouth and throat with clear clean water.

SKIN: Wash with clear clean water.

INHALATION: Remove from exposure.

EYES: Flush with quantities of water.

VI. REACTIVITY DATA

UNSTABLE: STABLE: X CONDITIONS TO AVOID: None known

INCOMPATIBILITY (MATERIALS TO AVOID): None known

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: None known

HAZARDOUS POLYMERIZATION:

MAY OCCUR: WILL NOT OCCUR: X CONDITIONS TO AVOID: None known

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED: Dike and absorb spill with inert material (kitty litter, etc) and transfer to suitable container for disposal.

WASTE DISPOSAL METHOD: Waste disposal depends upon local requirements. Assure adherence to Federal, State and Local regulations subsequent to disposal.

VIII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION: None required under usual conditions of use. However, if exposure potential exists refer to NIOSH Criteria Guides to determine appropriate unit.

VENTILATION: Local exhaust as necessary to reduce, prevent, and control aerosol generation at source.

PROTECTIVE GLOVES: Rubber/neoprene

EYE PROTECTION: Safety glasses or goggles

OTHER PROTECTIVE EQUIPMENT: As needed to prevent personal contact.

IX. SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING: Maintain good housekeeping, vacuum spills, avoid creating aerosol.

OTHER PRECAUTIONS: Long exposure of product to high heat and humidity may reduce product activity.

EPA REGISTRATION NUMBER: 58998-7

Foray™ 48B

Flowable Concentrate

KEEP OUT OF REACH OF CHILDREN

CAUTION

If in eyes, flush with plenty of water. Get medical attention if irritation persists.

ACTIVE INGREDIENT:	*Bacillus thuringiensis* Berliner var. *kurstaki*, primary powder fermentation product, potency of 12,600 IU per mg of product (equivalent to 48 Billion IU/gal.). 15%
	Inert Ingredient 85%
	Total 100%

DIRECTIONS FOR USE:

It is a violation of federal law to use this product in a manner inconsistent with its labelling. FORAY 48B contains the spores and endotoxin crystals of *Bacillus thuringiensis kurstaki.* is a stomach poison and has high specific activity against lepidopterous larvae. After ingestion, larvae stop feeding within hours and die 2-5 days later. Maximum activity is exhibited against early instar larvae. FORAY 48B Flowable Concentrate may be used for both ground and aerial application. The product should be shaken or stirred before use. Add some water to the mix tank, pour the recommended amount of FORAY 48B into the tank and then add the remaining amount of water to obtain the proper mix ratio. Agitate as necessary to maintain the suspension. The diluted mix should be used within 72 hours

Ground Application. Use an adequate amount of tank mix to obtain thorough coverage without excessive run off. Use the recommended per acre dosages of FORAY 48B in the following amounts of water:

High volume hydraulic sprayers	100 gallons
Mist blowers	10 gallons

Aerial Application: FORAY 48B may be applied aerially, either alone or diluted with water, at the dosages shown in the application rates table. Spray volumes of 32-128 ounces per acre are recommended. Best results are expected when FORAY 48B is applied to dry foliage

RE-ENTRY: FORAY 48B may be applied up to and including the day of harvest and in storage

STORAGE AND DISPOSAL: Do not contaminate water, food or feed by storage or disposal of waste.

Storage: Store in a cool, dry place. Keep containers tightly closed when not in use. Store in temperatures above freezing and below 30°C (90°F).

Pesticide Disposal: Pesticide waste resulting from the use of this product may be disposed of on site or at an approved waste disposal facility in accordance with federal and local regulations.

Container Disposal: Triple rinse for equivalent, then offer for recycling or reconditioning; or puncture and dispose of in a sanitary landfill, or by incineration or, if allowed by state and local authorities, by burning.
If burned, stay out of smoke.

PRECAUTIONARY STATEMENTS: Hazardous to humans: May cause eye irritation. Avoid contact with skin, eyes, open wounds, or clothing. Wash thoroughly with soap and water after handling.

Environmental Hazards: Do not contaminate water by cleaning equipment or disposal of waste.

WARRANTY NOTICE: All goods supplied by Novo BioKontrol are of high grade and we believe them suitable for the purpose recommended but, as we cannot exercise control over their storage or use, no responsibility will be accepted by us for any damage or injury whatsoever arising from their storage, handling, application or use

APPLICATION RATES.

Crop	**Pests**	Rate* (pts/acre)	Dosage* (BIU/Acre)
Forests, Shade Trees Ornamenals, Shrubs, Sugar Maple Trees	Gypsy moth, spruce budworm browntail moth	1-1/3 - 3-1/3	8 - 20
	Tussock moths, pine butterfly bagworm, leafrollers, tortrix mimosa webworm, tent caterpillar, jack pine budworm, black headed budworm, elm spanworm, saddled prominent saddleback caterpillar	1 - 2	6 - 12
	Red humped caterpillar, spring and fall cankerworm, california oakworm, fall webworm	05 - 10	3 - 6

*Use the higher recommended dose rate on advanced larval stages or under high density larval populations.

EPA Registration No: 58998-7
EPA Est No: 58998-DN-001

DISTRIBUTED BY:
Novo BioKontrol
A DIVISION OF NOVO LABORATORIES, INC.
33 Turner Road
Danbury, CT 06810-5101
(203) 790-2600

NET CONTENT **2.5**
(U.S. GALLONS)

Novo Nordisk
Entotech, Inc.
MEMO

TO: Jack Barry

CC:

FROM: DaH

DATE: April 18, 1991

RE: Forest Service - Drift Trials

Jack Barry
USDA Forest Service
Forest Pest Management
2121 C Second Street
Davis CA 95616

(916)-758-4600
-8181 (FAX)

Per your request the medium formula is as follows:

for one liter of formula:

Difco™ agar	15	grams
Difco ™ Nutrient Broth	8	
water	one liter	

No pH adjustment is needed.
Autoclave for 20 minutes at 121°C and 15 lbs. pressure.
Pour immediately into petri dishes.

After the plates have been exposed, growth can occur at room temperature or for faster growth, incubated at 30°C. Plates should be read in 12 - 48 hours.
Note: run a check plate to help identify Bt colonies, against contaminants.

The one liter sample can be picked up at your convenience, just call ahead to make sure I'm around.

David Hobbs

MESSAGE DISPLAY

T- Barry, Jack:SCS06

telephone (503) 750-7363

From: Roy C. Beckwith:S26L05A
Postmark: Apr 18,91 3:12 PM Delivered: Apr 18,91 3:18 PM
Status: Certified
Subject: Reply to: Culturing B.t.

Reply text:
From: Roy C. Beckwith:S26L05A
Date: Apr 18,91 3:12 PM

Jack--Here is the technique we use for needles--it should work for the wash from your impingement device. We use Tryptic Soy Agar for the media--the directions are on the box. Since the agar is autoclaved before use and we leave it for a short time-- we don't use any additives. I have never used the technique on Foray 48B; however, it is based on the HD-1 strain so should be the same as others we have used.

The technique is as follows:

1. Place 20 needles into a sterile test tube.
2. Add 20 ml of sterile distilled water.
3. Mix for 60 seconds.
4. Pour water into sterile test tube.
5. Make 1: 100 dilution (1 ml: 99 ml)
6. Mix for 60 seconds
7. Add 1 ml of dilute to sterile petri dish
8. Add about 10 ml of nutrient agar and mix contents by rotary motion (Agar is cool enough to be handled)
9. After agar gels, invert petri dish.
10. Leave petri dishes at room temperature from 24-48 hours.
11. Make spore counts (based on colonies) using a colony counter.

Preceding message:
From: Barry, Jack:SCS06
Date: Apr 18,91 1:39 PM

Roy, we are conducting a drift study in cooperation with R-4's gypsy moth project. Foray 48B will be used and we plan to use impingers to collect aerosols of the sample. The sampler effluent will be plated out to get a total count per unit of air sampled. What I need is your recommendation of growth media recipes that would be best suited for Foray and any additives that we might need to add to inhibit growth of contaminants. And any other thoughts. The Dugway Proving Ground lab will be doing this for us and they have a lot of

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COLLECTION EFFICIENCY OF ROTOROD SAMPLERS FOR SAMPLING FUNGUS SPORES IN THE ATMOSPHERE

Robert L. Edmonds[1]

Abstract

In the sampling of fungus spores in the atmosphere, the collection efficiency, and thus the accuracy of the samplers for obtaining quantitative data, has rarely been considered for the particular fungus spore being sampled. This paper is designed to make potential users of Rotorod impaction aerosol samplers aware of the importance of considering sampling efficiency. A method for calculating efficiency is given.

Adequate study of the dispersion of fungus spores requires accurate sampling of the atmosphere. In many investigations the collection efficiency, and thus the accuracy of samplers, has not been considered for the particular type of fungus spore being sampled.

Suction devices and rotating arm impactors are the most common types of instruments used in the collection of fungus spores. Because of the large size range of fungus spores (from a few microns (μ) to 100μ), however, there is no one instrument yet developed that is capable of sampling the whole range with equal efficiency.

This paper proposes to indicate to users of rotating arm impaction samplers the importance of considering collection efficiency in sampling; to discuss theoretical aspects in determination of efficiencies; to provide an equation for determination of sampling efficiencies for various sizes of fungus spores; to demonstrate how collecting surfaces can be modified to in-

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[1] The author is presently Program Coordinator, United States International Biological Program, Aerobiology Program, Botany Department, University of Michigan, Ann Arbor, Michigan 48104.

crease sampling efficiency; and to discuss the importance of the selection of a suitable sticky material for the leading edge of the sampler. The "Rotorod sampler" is used as an example of this type of device. It is commercially available and widely used.

The "Rotorod sampler" in the form developed and marketed by Metronics Associates, Inc. of Palo Alto, California (10) has been used for collecting fungus spores in the atmosphere by many workers including Asai (1), Froyd (5), Barksdale (2), Skilling (9), and Edmonds (4). It employs the process of inertial impaction with spores, and so forth, being impacted on a whirling arm.

Advantages of this sampler are low cost, simplicity, light weight, large sampling volume, suitability for experiments employing large numbers of simultaneous samplers, battery operation for remote locations, and the collection efficiency is not affected by wind speed up to 6.2 kph. The chief disadvantage is that collection efficiency is sharply dependent on spore size and density, and it can only be used for short periods of time because of over-loading of the collection surface.

Two sizes of Rotorods are available commercially from Metronics (Fig. 1). The U-shaped brass Rotorod has collection surfaces 1.59 mm in thickness, with arms 6 cm high, 8 cm apart and samples 120 liters per minute (lpm). It was designed to sample particles in the 15-25μ diameter range. The H-shaped chromel Rotorod is 0.38 mm in thickness, with arms 6 cm high, 12 cm apart and samples 41.3 liters per minute. It was designed to sample fluorescent particles (specific gravity 4.0 g cm^{-3}) in the 1-5μ diameter range (10). The Rotorod motors revolve at approximately 2400 rpm, with the collecting surfaces of the U- and H-shaped Rotorods revolving at 15.1 and 10.1 m sec^{-1}, respectively. The actual rpm for each motor varies and is supplied for each motor.

FIGURE 1. Rotorod samplers and motors. Commercially available Rotorods are the U-shaped (right) and H-shaped (center). Modified Rotorod is on the left.

DISCUSSION

Most researchers who are interested in collecting airborne spores usually wish to know the spore concentration in the atmosphere. Erroneous spore concentrations, however, can be calculated if collection efficiencies are not considered for the particular spore size under consideration. Not all spore sizes, even within the suggested range of the instrument, are collected with equal efficiency. Sampling sensitivity is low if efficiency is low, and low efficiencies also result in uneven distributions of spores on the collection surfaces (8). This is important to consider if sample fields are to be counted on the Rotorod arms.

Noll (8) has discussed theoretical and experimental aspects of whirling arm samplers. The collection efficiency (E) is largely a function of the particle parameter (P) (Fig. 2). Experimental data in this figure were obtained from a 16-stage rectangular collector impaction sampler developed by Noll. Each stage was designed to sample a specific size range of particles at 85-100% efficiency. The particular data used were derived from two stages designed to collect particles down to 26 and 13μ respectively. These stages had collection surfaces 3.2 and 0.8 mm in width, respectively, and revolved at 7.2 m sec^{-1}. Noll's data generally agree with experimental data of Chamberlain and Gregory for impaction of Lycopodium spores on cylinders (3). The efficiency suggested by Chamberlain for P equal to 10, however, is lower than those values suggested by Noll. Noll's data are preferred in practice because they are derived from a whirling arm sampler similar to the Rotorod, with similar sized rectangular collection surfaces, revolving at similar velocities. The line through the data was drawn as the line of best fit by eye.

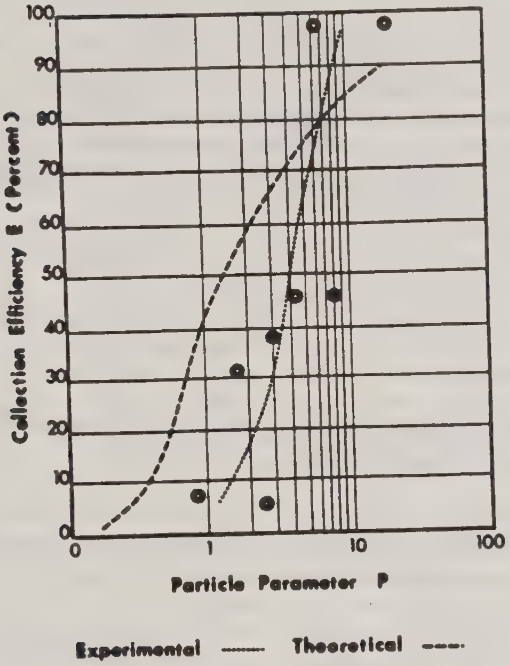

FIGURE 2. Relationship between particle parameter (P) and spore collection efficiency (E). (After (8)).

The theoretical curve presented in Figure 2 was determined by Langmuir and Blodgett for flow around a ribbon (8). Experimental values of E are lower than theoretically derived values, for P less than 7, but higher for P greater than 7. Chamberlain noted that experimental values of E were always lower than theoretical. He is uncertain whether this represents a fault in theory or a failure by sticky cylinders to retain all spores striking them. It would appear that the desired efficiency of 100% is approached as P approaches values of 10 or greater. In practice, because of the inconsistent agreement with theory, it is preferable to use the experimentally derived curve to determine E.

The selection of a suitable sticky material for the leading edge is important. If the surface is dry, particles bounce off. The material must be sticky, but if it is too thin, friction causes it to run off. If it is too thick, the edge loses its sharpness, the effective size of the collection surface is increased, and the collection efficiency is lowered. A 1:3 rubber cement and xylene solution used by Harrington, et al. (7), Froyd (5), and Edmonds (4) appears to give reasonable results.

The following is a general formula to calculate P for any spore size:

$$P = \frac{V_o d^2 \delta_p}{18 \eta L S}$$

P = Particle Parameter (dimensionless)
V_o = Average* Rotorod arm velocity (cm sec^{-1} U-shaped (1010)
H-shaped (1510)
d = Diameter of sphere of equivalent volume to that calculated for the spore (cm)
δp = Density of spore (g cm^{-3})
n = Viscosity of air (poises, g sec^{-1} cm^{-1}), at 18°C = 182.7×10^{-6} poises
L = Width of rectangular collector (cm)
S = Dynamic shape factor of particle (dimensionless)

*Actual arm velocity is variable because rpm vary from motor to motor.
Actual rpm for each motor is supplied by manufacturer.
The value of E is read from Figure 2.

Fuchs (6) has suggested that S is 1.28 for ellipsoids with ratio of axes, major/minor = 4. For practical purposes, no great error is made by setting S = 1 for spores with ratio of axes less than 4.

Table 1. Values of particle parameter (P) and collection efficiency (E) of U-shaped Rotorods in spore sampling studies.

Author	Organism	Type of spore[a]	Average dimensions of spore (μ)	Diameter of spherical spore of equivalent volume (μ)	P particle parameter (from formula)	E Percent (from Figure 2)
Asai (1)	*Puccinia graminis*	Uredospore	24 x 18.5	22	9.4	95
Froyd (5)	*Hypoxylon pruinatum*	Ascospore	26 x 10.5	19	7.0	80
Barksdale (2)	*Piricularia oryzae*	Conidia	23 x 8.5	16	4.9	65
Skilling (9)	*Scleroderris lagerbergii*	Ascospore	19.5 x 5	12	2.8	30

[a] Spore density was assumed to be 1.0 and S = 1 (ratio of axes of spores is less than 4). Diameter of equivalent sphere is rounded to nearest whole number.

Asai, Froyd, Barksdale, and Skilling used U-shaped Rotorods in their experiments. Asai, however, was the only investigator to mention sampling efficiency, noting that spores in the vicinity of 20μ diameter are impacted at approximately 100% efficiency.

Table 1 shows values of P and E for each of the four studies. The *Puccinia graminis* spores trapped by Asai are impacted at close to 100% efficiency. The other spores are sampled at much lower efficiencies. Unit densities for spores was assumed.

The H-shaped Rotorods will impact spores of unit density down to 9μ in diameter at close to 100% efficiency. Thus, in selection of Rotorod size to be used, it is important that sampling efficiencies for both sizes of Rotorods be determined for the particular spore in question, in order to obtain maximum efficiency. If P is greater than or equal to 10, the efficiencies close to 100% can be obtained.

H-shaped Rotorods can also be modified (Fig. 1) to a particular efficiency by welding aluminum shims of different widths to the arms, thereby adjusting the width of the collection surface and increasing the collection efficiency. Welding of shims to arms increases the aerodynamic drag on the rotating arms, which probably results in small decreases in the rotation speed of the sampler perhaps of 5% or so. This was not checked in practice, but it should be considered. This modification shown in Figure 1 was used by the author to collect spores of *Fomes annosus* (4.5-5.0μ diameter)(4) at an efficiency similar to that of fluorescent particles (specific gravity 4.0 g cm^{-3}, 3.0μ average diameter) collected on H-shaped Rotorods. This enabled a direct comparison of their respective dispersion patterns, with the object of determining if fluorescent particles could be used for tracing spore dispersal.

Another factor to be considered in collection efficiency is that of possible changes in shapes and, thus effective sizes of fungus spores while they are airborne due to changes in moisture content, and so forth. This is difficult to assess and thus has not been considered in the calculations.

CONCLUSION

In sampling fungus spores with a rotating arm impaction device such as the Rotorod, it is important to consider the collection efficiency for the particular species of fungus spore being sampled in order to make accurate calculations of the concentration in the atmosphere. If the particle parameter P is calculated to be greater than 10, then the efficiency of collection is close to 100%. If P is less than 10, then the collection efficiency can be read from Figure 2.

An alternative to this is to modify the size of the collecting surface to increase collection efficiency.

Literature Cited

1. ASAI, G. N. 1960. Intra- and inter-regional movement of uredospores of black stem rust in the Upper Mississippi River Valley. Phytopathology 50: 535-541.
2. BARKSDALE, T. H. 1967. Spread of rice blast in small fields. Plant Disease Reptr. 51: 243-247.

3. CHAMBERLAIN, A. C. 1967. Deposition of particles to natural surfaces. *In* Airborne Microbes. P. H. Gregory and J. L. Monteith (Ed.). Cambridge University Press. pp. 138-164.
4. EDMONDS, R. L. 1971. Dispersion and deposition of spores of Fomes annosus and fluorescent particles into and within a forested area. Ph.D. Thesis. University of Washington. 171 pp.
5. FROYD, J. D. 1964. Studies of the biology of hypoxylon canker of aspen. M.S. Thesis. University of Minnesota, Minneapolis. 80 pp.
6. FUCHS, N. A. 1964. The mechanics of aerosols. Permagon Press, New York.
7. HARRINGTON, J. B., G. C. GILL, and B. R. WARR. 1959. High efficiency pollen samplers for use in clinical allergy. J. Allergy 30: 357-375.
8. NOLL, K. E. 1970. A rotary inertial impactor for sampling giant particles in the atmosphere. Atmospheric Environment 4: 9-20.
9. SKILLING, D. D. 1969. Spore dispersal by Scleroderris lagerbergii under nursery and plantation conditions. Plant Disease Reptr. 53: 291-295.
10. WEBSTER, F. X. 1963. Collection efficiency of the Rotorod sampler. Tech. Rept. No. 98. Metronics Assoc., Palo Alto, California AD 48 597.

UNITED STATES INTERNATIONAL BIOLOGICAL PROGRAM, AEROBIOLOGY PROGRAM, BOTANY DEPARTMENT, UNIVERSITY OF MICHIGAN, ANN ARBOR, MICHIGAN

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A QUANTITATIVE SAMPLING METHOD FOR AIRBORNE SWEET CORN POLLEN UNDER FIELD CONDITIONS[1]

P. K. FLOTTUM, D. C. ROBACKER, AND
E. H. ERICKSON, JR.[2]

Abstract

The rate of pollen dehiscence in a sweet corn (*Zea mays* L.) plot was measured using a Rotorod Sample®. Samples were taken from 0700 to 1230 h for 3 days during anthesis. Totals were averaged over the 3 days, and the resulting composite data were used to develop a sampling protocol accurate for determining the rate of pollen release. Results showed that a 10-min sampling period, with a frequency of at least once every half hour was required to accurately reflect the pollen release rate.

Additional index words: **Pollen, Dehiscence, Pollen emission profile, *Zea mays* L.**

STUDIES of pollen dehiscence in sweet corn (*Zea mays* L.), and many other grasses (Gramineae) have been primarily concerned with developing techniques that predict the date flowering will begin (Cross and Zuber, 1972; Gardner et al., 1981; Hanway 1966). In sweet corn the process of pollen dehiscence, usually defined only as anther decention, is fairly well understood (Knox, 1979; Percival, 1969). However, studies of patterns of pollen release for a single day or for the period of anthesis have generally been qualitative in nature, as quantitative in vivo measurements of airborne sweet corn pollen have not been made.

This paper 1) describes a method used to measure the pollen emission patterns in a flowering sweet corn field, 2) documents the effectiveness of the method, and 3) presents data pertinent to the optimal use of the method.

Materials and Methods

A Rotorod Sampler®[3], a rotating impaction device powered by a 12 v battery, was used to collect airborne sweet corn pollen. Airborne pollen is captured on the leading surface of removeable 64-mm plastic rods held by the rotating arms (Fig. 1). General Electric G-697 Silicone Grease®[3] facilitates capture and retention of the pollen grains. The arms are rotated at ca 2400 RPM, sampling a volume of ca. 120 L/min. Operating efficiency, or ability to collect airborne pollen in the volume sampled, was determined to be greater than 99% by manufacturers specifications.

These studies were conducted at Madison, Wis. during 1981. The Rotorod Sampler was positioned centrally in a 40 m^2 plot of 'Commander' sweet corn with the maximum height of the sampler rods slightly below center of representative tassels in the plot. Sample rods were replaced at the end of each sampling interval whereupon the number of captured pollen grains were counted.

Fig. 1. The Rotorod Sampler®, showing removable 64 mm plastic rods.

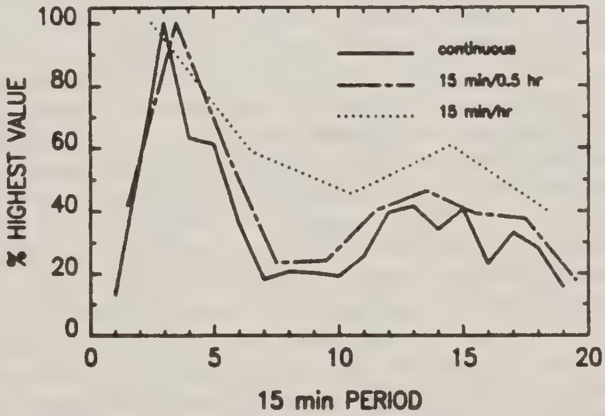

Fig. 2. Relationship of continuous, 1 sample/h and 1 sample/0.5 h profiles.

Two concurrent collection procedures were designed and their results compared. The first procedure, subsequently referred to as the continuous method, consisted of running one Rotorod Sampler for 22 consecutive 15 min periods, from 0700 to 1230 h, for 3 days in the flowering sweet corn plot. Actual numbers of collected pollen grains were converted to pollen grains/L and plotted by period to develop a pollen emission profile for each day. The three daily profiles were then aligned so that the 15-min periods containing the initial daily peak coincided. This was done in order to accommodate the relative times of pollen dehiscence, not absolute time of day. Initial daily samples containing no pollen were not included in the analysis. Thus, the resulting composite profile consisted of 19, 15-min periods (Fig. 2).

To determine if reliable data could be obtained from fewer sampling periods than the 19 used to develop the composite of the continuous sampling method, the same data were reanalyzed assuming 1) one 15-min sample/h and; 2) one 15-min sample/0.5 h. For the one sample/h method, three groups of 15-min periods were analyzed as

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[2] Specialist and research associate, respectively. Dep. of Entomology, Univ. of Wisconsin, Madison; and professor, USDA-ARS, Bee Res. Unit, Dep. of Entomology, Univ. of Wisconsin, Madison, WI 53706.

[3] Ted Brown Assoc., 26338 Esperanza Dr., Los Altos Hills, CA 94022. Mention of a trade name does not constitute a guarantee or warranty of the product by the USDA nor an endorsement over other products not mentioned.

if each group represented a distinct replication of the method. From Fig. 2, nonrandomized groups used were:

Group 1: periods 1,5,9,13, and 17
Group 2: periods 2,6,10,14, and 18
Group 3: periods 3,7,11,15, and 19

For the one sample/0.5 h, two nonrandomized groups were analyzed as distinct replications:

Group 4: periods 1,3,5,7,9,11,13,15,17, and 19
Group 5: periods 2,4,6,8,10,12,14,16,18, and 19[4]

Regressions of pollen emission on period were conducted for each of the three data arrangements (continuous, one 15 min sample/h and one 15 min sample/0.5 h) using orthogonal polynomials to represent period numbers.

The second collection procedure used consisted of running another Rotorod Sample® for a period of only 10 min/sample. Samples were collected for the first 10 min of periods 1,3,5,7,9,11,13,15,17, and 19, (one 10 min sample/0.5 h), with the sampler left idle for the remaining 5 min of the period. These samples were neither randomized nor replicated. Results of these 10-min samples were analyzed as above and compared to the composite results and to the results of the one 15-min sample/0.5 h.

Results and Discussion

Method 1

Daily pollen emission profiles were strikingly similar. A large peak was recorded during one of the first 3 15-min periods each day. This was followed by a reduction in emission intensity, another slight increase then decreasing thereafter.

Regression analyses to determine the relationship between the amount of pollen collected and the time of day demonstrated significant linear ($P < 0.05$), quartic ($P < 0.01$), and quintic ($P < 0.05$) coefficients for each of the three daily profiles and the composite.

Regression coefficients for the one sample/0.5 h method, and for the composite profile (continuous method) were not significantly different (linear = -0.01 vs. -0.01; quartic = -0.004 vs. -0.002; quintic = 0.005 vs. 0.001, respectively - regression coefficients not converted from orthogonal polynomials). Similar analyses for the one sample/h method showed linear significance ($P < 0.05$), but neither the quartic nor the quintic coefficients were significant. Therefore, by inspection and analyses interpretation, it is shown that the curves for the continuous and one sample/0.5 h data arrangements are clearly high degree polynomials, while the curve for the one sample/h data arrangement is only a first degree relationship. These relationships are represented graphically in Fig. 2. Had sampling begun earlier each day, the initial peak may have been evident in the one sample/h arrangement, but this would not alter the lack of significance for the second peak.

Method 2

Results of the second technique, running the sampler for a period of 10 min/0.5 h, were compared to the results of the continuous method and to those of the one 15 min sample/0.5 h data arrangement. These were not significantly different at the levels previously noted as there were no differences in the rate of pollen collection or shapes of the profile curves. Hence, one 10 min sample/0.5 h retained the accuracy of continuous sampling and displayed the bimodal emission profile of the sweet corn population. Further, this sampling frequency optimized accuracy of the data and reduced the effort required in data acquisition. Moreover, it permits one operator to gather data simultaneously from several locations in a large field.

Pollen release is dependent on several environmental variables (Flottum et al., 1983), as is the amount of pollen collected on the sampler. For this reason, use of the sampler cannot accurately determine when anther decention and subsequent pore formation occurs, but rather when the released pollen becomes airborne. In spite of this, the Rotorod Sampler®, when used in the manner described, is an accurate method of recording the pollen emission patterns in a sweet corn field.

References

1. Cross, H.Z., and M.S. Zuber. 1972. Prediction of flowering dates in maize based on different methods of estimating thermal units. Agron. J. 64:351–355.
2. Flottum, P.K., E.H. Erickson, and B.J. Hanny. 1983. The honey bee-sweet corn relationship: Am. Bee J. 123(4):293–299.
3. Gardner, B.R., B.L. Blad, R.E. Maurer, and D.G. Watts. 1981. Relationship between crop temperature and the physiological and phenological development of differentially irrigated corn. Agron. J. 73:743–747.
4. Hanway, J.J. 1966. Corn plant development. Special Report no. 48 Science and Tech. Coop. Ext. Service, Iowa State Univ., Ames.
5. Knox, R.B. 1979. Pollen and Allergy. Univ. Park Press, Baltimore, Md. 59p.
6. Percival, M.S. 1969. Floral Biology. Pergamon Press, New York 243p.

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[4] Nineteen used again for balance.

FIELD CREW CHECK SHEETS AND REPORT

CREW: ______________________________

TRIAL: ______________________________

DATE: ______________________________

A. Inventory	Qty Required	Qty Picked-up	Remarks
B/C Unit			
Nicad Battery w/cabel			
Tubing			
Orifices 12.5 lpm			
Wagners			
Rotorod Motors			
Rotorod Holders			
Rotorods			

STEDP-MT-L-A(ROME) 25 SEPTEMBER 1991

MEMORANDUM FOR U.S.D.A. FOREST SERVICE (JACK BERRY)

SUBJECT: SOP FOR ASSAY OPERATIONS /MICROBIOLOGY LABORATORY

1. ENCLOSED IS THE SOP WE DISCUSSED. THIS SOP ALSO HAS THE FORMULA FOR THE PLATE MEDIUM THAT WE USED WITH THE BT ON PAGE 9.

2. THANKS FOR THE WORK. WE ARE LOOKING FORWARD TO DOING THIS AGAIN NEXT FALL.

WILLARD ROME

MT-L 389 2nd Revision

LIFE SCIENCES DIVISION/BIO ASSAY BRANCH 20 OCT 1987

ASSAY PROCEDURES FOR BIOLOGICAL
SIMULANTS/~~PREPARATION AND SAMPLER~~
SAMPLER Preparation

DTC SOP 70-100

CHIEF, ASSAY BRANCH

I. RESPONSIBILITIES:

A. Will provide training and supervision for personnel assigned to various laboratory duties.

B. Will assure compliance with this SOP.

MICROBIOLOGIST IN CHARGE

II. RESPONSIBILITIES:

A. Will understand and implement the procedures outlined in this SOP.

B. Will be responsible for producing simulant in quantities needed to support field and laboratory testing, and for quality control of simulant in accordance with test plan criteria.

C. Will maintain continuous surveillance of simulant storage conditions and techniques, and field and laboratory sampling procedures to verify they are consistent with sound quality control and microbiological procedures.

MICROBIOLOGIST AND TECHNICIANS

III. LABORATORY PROCEDURES:

A. PREPARATION AND ASSESSMENT OF THE WAGNER SAMPLER.

1. Preparation of the Wagner Sampler: The vacuum stem and sampling stem of clean Wagner samplers will be plugged with non-absorbent cotton, sterilized and dried in a sterilizer. Samplers are then removed and

cooled. In a clean area, the samplers are taken apart and sterile filters are inserted over the sampler mat using a pair of sterile forceps. The forceps are "flamed off" at regular intervals to prevent contamination. The samplers are reassembled with identification tags attached and the appropriate number of samplers for each station placed in a carrier. The carrier is identified as to crew and station. The samplers are then ready for storage or for distribution.

2. Assay of the Wagner Sampler: After the Wagner samplers have been returned from the field, the sampler will be broken down and, with the aid of sterile forceps, the filter and the mat removed and placed into sterile 50ml screwcap tubes contianing 10ml of gelatin phosphate diluent. The tube is then identified as to the station number and sampler sequence. The forceps are flamed between samples to prevent cross contamination. The samples are then shaken for 10 minutes on a mechanical shaker to suspend the BG prior to assay.

3. Preparation for Assay (i.e. plating): The appropriate number of casitone media plates are removed from refrigerated storage and set out at room temperature before plating begins in order to allow any condensate to dry off. An adequate supply of the following equipment is required:

(a) 9ml dilution blanks with the appropriate diluting fluid.

(b) Sterile 1.0ml pipettes graduated in 0.01ml.

(c) Sterile spreaders

(d) Pipette and spreader discard pans

(e) Felt tip marking pens

A disinfectant (i.e. 70% alcohol) will be kept available for disinfecting spilled materials and surfaces.

4. Assay Technique: The plating crew will consist of two persons. One person will act

as the "spreader" and will be responsible for marking plates with the necessary identification and spreading the inoculum on the plates; the other person will act as the pipettor and is responsible for making dilutions and placing the inoculum on the plates.

(a) The "spreader" first checks the plates for contamination and then numbers the plates with the sample number and dilution. Duplicate plates are made on each dilution. Undilute plates are marked with a "U". Consecutive dilutions are numbered 1,2,3, etc. The plates are then passed to the pipettor.

(b) The pipettor mixes the undiluted sample by using a laboratory test tube vortex mixer swirlling the liquid up the tube ten times. He then uses a 1.0ml pipette and draws up 1.0ml of fluid, then touches the tip of the pipette to the inner wall of the tube to remove any excess fluid. While holding the pipette at an angle of about 30 degrees, and with the tip of the pipette resting on the agar surface, 0.2ml is delivered to the plate marked "U". When the inoculum is delivered, the pipette is touched on a dry portion of the agar and drawn through a one-inch arch to remove fluid adhering to the tip. This procedure is repeated on the duplicate plate.

(c) The plates marked "U" are then passed back to the "spreader" to be spread.

(d) Using the same pipette, the pipettor again draws 1.0ml of the fluid from the undiluted sample. The contents of the pipette are then delivered to a 9ml dilution blank. The pipette is then placed into the pipette discard pan. The tube is mixed as before. Using a clean sterile pipette, the fluid is drawn and plated using the same technique as the undiluted sample. This plate will be the U+1 dilution (i.e. 1:10). This procedure is repeated for all subsequent dilutions required, changing pipettes between each dilution before mixing. The number of dilutions and the dilutions to be plated are determined beforehand. When a higher concentration of organisms is suspected, more dilutions are made, and the lower dilutions are not usually plated in order to conserve time and material.

(e) After the inoculum has been delivered on a plate, they are passed to the "spreader". The "spreader" takes a clean, sterile spreader and spreads the fluid by using a circular motion, starting in the center of the plate and working toward the edge. Care is taken to keep the fluid from being thrown upon the sides of the plate, and a straight movement across the plate may be necessary for uniform distribution of the inoculum over the entire surface of the agar. The spreader is kept in contact with the agar surface at all times. Agar plates containing bubbles or contaminating growth or too thin to support growth are discarded before plating. Used spreaders are placed into the pipette discard pan.

(f) All undilute samples and dilutions are stored at 4°C until data are obtained for each sampling station. (Re-assay may be required in cases of missed dilutions or challenged estimates.) If an accident should occur resulting in the spillage of sample, the contaminated area is flooded with 70% alcohol and wiped up to prevent contmination of other equipment.

(g) Immediately after the assay of the samples is completed, the identification tags or tapes are removed from the tubes and samplers before sterilizing and all used glassware and equipment or that which has come in contact with potentially contaminated material is sterilized. The plating area is then washed down with 70% alcohol.

(h) The spread plates are placed in a pan by the "spreader" right side up, one sample per stack, with the undilute plates on the bottom and the highest dilution on top, and the inoculum allowed to dry. When the inoculum has soaked into the medium on the last plate spread, the plates are then inverted and placed on the incubator shelves.

Plates are incubated at 37°C and require 18 to 24 hours to develop countable colonies.

5. Counting Plates: All plates that have 300 or less colonies are counted except in cases where the highest dilution has more than 300 colonies or when the plates are not countable due to contamination or plating error. In these cases, an estimate of the count may have to be made on the next countable dilution. If it is not feasible to count all of the colonies, the plate is divided into sections, by aid of guidelines on the counter, and one or more sections are counted and the count multiplied by the correct factor in order to obtain an estimate of the total count. After plates have been counted, they are sterilized, Prior to sterilization the plates are placed in pans and covered with water. A small quantity of detergent is added to the water. After sterilization, the melted agar is washed down the drain and the melted plates are placed in plastic garbage bags and disposed of in a dumpster.

B. PREPARATION AND ASSAY OF THE REYNIER SLIT SAMPLER: The Reynier sampler uses a clock, which may be either AC or DC current, to sample air containing a biological aerosol over a period of time from 1 minute to 2 hours depending on the Reynier sampler used.

1. Preparation of the Reynier Slip Sampler:

(a) Slit Adjustment: The width of the slit opening will be set at 0.006 inch when using the sampler at a sampling rate of 1 cubic foot per minute. This is done by loosening one of the screws in the slit assembly and inserting a 0.006 inch leaf type feeler guage. By holding the slit assembly tight against the feeler guage and tightening the screw, the slit is adjusted for the correct clearance.

(b) Cleaning Slits: Slits are cleaned with a one-half inch brush to remove all debris in the slit area. A clean brush is then dipped in 70% alcohol and the complete slit area, including the undersurface, brushed off and dried.

(c) Timer adjustment: The timer is set by rotating the petri plate retainer clockwise until the pointer is on or past the zero mark. Turn the clock release on for a few seconds to insure proper running condition of the clock mechanism.

(d) Plate Installation: The appropriate plates containing agar are used. Remove the cover of the Reynier sampler exposing the plate retainer. Mark a starting line on the bottom of the petri plate with a permanent marker and install the plate on the retainer, lining up the mark on the plate with the zero mark on the clock. Replace and tighten cover.

(e) Slit Height Adjustment: Adjust the slit height by releasing and lowering the slit height guage until it rests lightly on the surface of the agar. Adjust the slit-to-agar surface distance by turning the slit tube knob until the pointer on the height guage corresponds to the 4mm mark on the tube scale. Raise and tighten the height guage with a counter-clockwise movement. Check to insure that the sampler identification tag is securely on the sampler.

2. Assay:

(a) At the conclusion of the sampling period the samplers will be returned to the laboratory and the plates removed after the external surfaces of the sampler have been wiped with 70% alcohol. The plate lids are replaced with the appropriate identification written on the lid. All plates are incubated at 37°C for 18-24 hours. After incubation, the plates are counted using the 12 or 6 degree segmented grid for the 2 hour Reynier clocks. Each segment corresponds to four or two minutes of sampling time respectively. The plates will be positioned so that the starting mark made on the plate will correspond to the "start" line on the counting grid.

(b) The number of colonies per segment will be counted. All colonies on the lines defining the left margin of each segment will be counted as belonging to the segment. If the segment contains too many colonies for an accurate count it will be marked "TNTC" (too numerous to count).

C. PREPARATION AND ASSAY OF THE ALL-GLASS IMPINGER (AGI) AND PRE-IMPINGER:

1. All-Glass Impinger:

(a) All inpingers will be calibrated at a flow rate of either 6.0 or 12.5 liters (l) per minute, as specified by the test operations plan.

(b) The impingers will be washed and thoroughly rinsed in distilled water. The clean, dry impinger tops and bottoms will be assembled, and the inlet and outlet tubes plugged with non-absorbent cotton and sterilized.

(c) The sterile impingers will be filled aseptically with the appropriate, sterile collecting fluid. Six l/minute impingers will have 18.5 ml of fluid. 12.5 l/minute impingers will have 20.0 ml of fluid.

(d) After filling the impingers, the tops will be secured to the bottoms by placing a rubber band around the neck at the bottom and slipping it over the outlet stem of the impinger top. Labels will be placed on the impinger for station identification.

(e) The impingers will be arranged in racks according to crew listing and will be numbered according to station number.

2. Pre-Impingers:

Dry, plastic pre-impingers usually will not be assayed. Each pre-impinger will have a piece of rubber tubing which is rigid enough to prevent the pre-impinger from sagging or drooping when connected to the impinger.

3. Assay of AGI Samples:

(a) Measuring volume of collecting fluid:

To calculate the number of colony forming units (CFU) collected, the volume of the fluid remaining in the impinger after operation must be determined. The contents of the impinger will be poured into graduated test cylinders and readings taken to the nearest 0.1ml. The adhesive labels bearing the number of the impinger will be transferred to the tube to label the sample. The volume of the impingers will be recorded.

(b) Plating will be done using the plating technique for the assay of the samplers.

D. PREPARE AND ASSAY OF THE 6-STAGE ANDERSEN SAMPLER:

1. The samplers should be inspected and free from dust and dirt. The holes in the 4th, 5th and 6th stages should be examined under a stereoscope (10X). Any plugged holes should be carefully punched out with a cleaning wire of appropriate diameter. When working with simulants, it is not necessary to sterilize the samplers. However, a 70% alcohol solution should be used to wipe the samplers between tests.

2. The Andersen plates (glass) will be brought to ambient temperature before using. The samplers will be loaded in a sanitary area by personnel dressed in clean clothes. Always start loading the sampler with six plates in a stack - numbered from six (bottom) to one (top). Putting hard pressure on the top stage of the Andersen, release the 3 spring fasteners. Remove the six stages of the sampler and begin loading by placing the No. 6 dish on the base and the No. 6 stage over it. The remaining plates and stages will be assembled in descending order. Putting hard pressure again on the top stage of the Andersen, hook the sampler fasteners, taking care to insure that the stage gaskets are in place. The sampler is then stoppered and marked for identification.

3. The Samplers will be placed in

containers and the containers identified with the appropriate station number.

4. After exposure, the samplers are collected and returned to the laboratory. The exterior of the sampler container is wiped with 70% alcohol and the sampler plates removed. The lists are replaced on the plate bottoms, the plates identified as to stage and sampler number, and incubated at 37°C.

5. After incubation (18-24 hours) the plates are counted. Data will be reported to the microbiologist in charge. The microbiologist will use the appropriate form to report the data.

E. PREPARATION OF DILUENT/COLLECTING FLUID: Gelatin phosphate is generally used as a diluent and collecting fluid for BG and prepared as follows:

NA_2PO_4..........	4.0g/l
Gelatin.........	2.0g/l
Deionized H_2O...	1 liter

Dissolve the ingredients in water by heating. Adjust the pH to 7.0 ± 0.1 with 5N HCl. Sterilize in an autoclave 15 psi for 20 minutes or longer depending on the size of the container and quantity being sterilized. Add Antifoam A (1ml of a 1:10 dilution per 1) to suppress foaming.

F. PLATES:

1. Preparation of Plating Medium: The plating medium for BG is generally Tryptose Agar and is prepared as follows:

Bacto-Casitone......	20 gms/l
Dextrose............	10 gms/l
Agar Agar...........	20 gms/l
Sodium chloride.....	5 gms/l
Green food coloring.	0.3 ml/l

Suspend the ingredients in cool water and stir while heating to a boil. After all ingredients are dissolved, adjust the pH to 6.9 ± 0.1 with 5N NaOH. Add actidione (10 ml of a 1% aqueous solution) for each liter of plating medium. At temperatures below freezing tryptose agar plating media is prepared as follows for Andersen or Reynier samplers:

Bacto-Tryptose.........	20 gms/l
Dextrose...............	10 gms/l
Agar Agar..............	15 gms/l
Sodium chloride........	5 gms/l
Green food coloring....	0.3 ml/l
Methyl cellulose (CMC).	25 gms/l

G. PREPARING PLATES:

1. Regular AGI and Wagner Sampler Plates: The media is prepared as above and cooled to the appropriate temperature. 20 mls of medium is dispensed aseptically into each sterile petri dish by using a calibrated Brewer pipetting machine. Plates are poured on a flat level surface that has been washed down with a solution of sodium or calcium hypochlorate (66 gms/gallon) and wiped dry before the plates are set out. If necessary, the plates may be stacked 5 or 6 high and poured from the bottom up to conserve space. After plates are poured and the medium has solidified, they will be inverted and allowed to dry and age for a minimum of 24 hours. Aged plates will be stored in a 4 degree C refrigerator until used.

2. Andersen Sampler Plates: Glass Andersen Sampler plates are poured using the same procedures outlined above except that 27 mls of medium is dispensed into each sterile glass Andersen Sampler plate.

3. Reynier Sampler Plates: Reynier Sampler plates are poured using the same general procedure as above except that 80 mls of liquid medium is dispensed

into each sterile plate and plates should never be stacked until the medium has solidified.

H. REPORTING DATA:

1. Data will be reported on the appropriate MT-L-A forms provided for that purpose.

2. The microbiologist in charge will review the data, explain inconsistencies if possible, and make duplicate copies, one copy for MT-L-A and one copy for the Project Officer.